Impact of target binding on the pharmacokinetics of small-molecule drugs: Insights via PBPK modeling (revisiting the case of warfarin)

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Target-mediated Drug Disposition (TMDD)

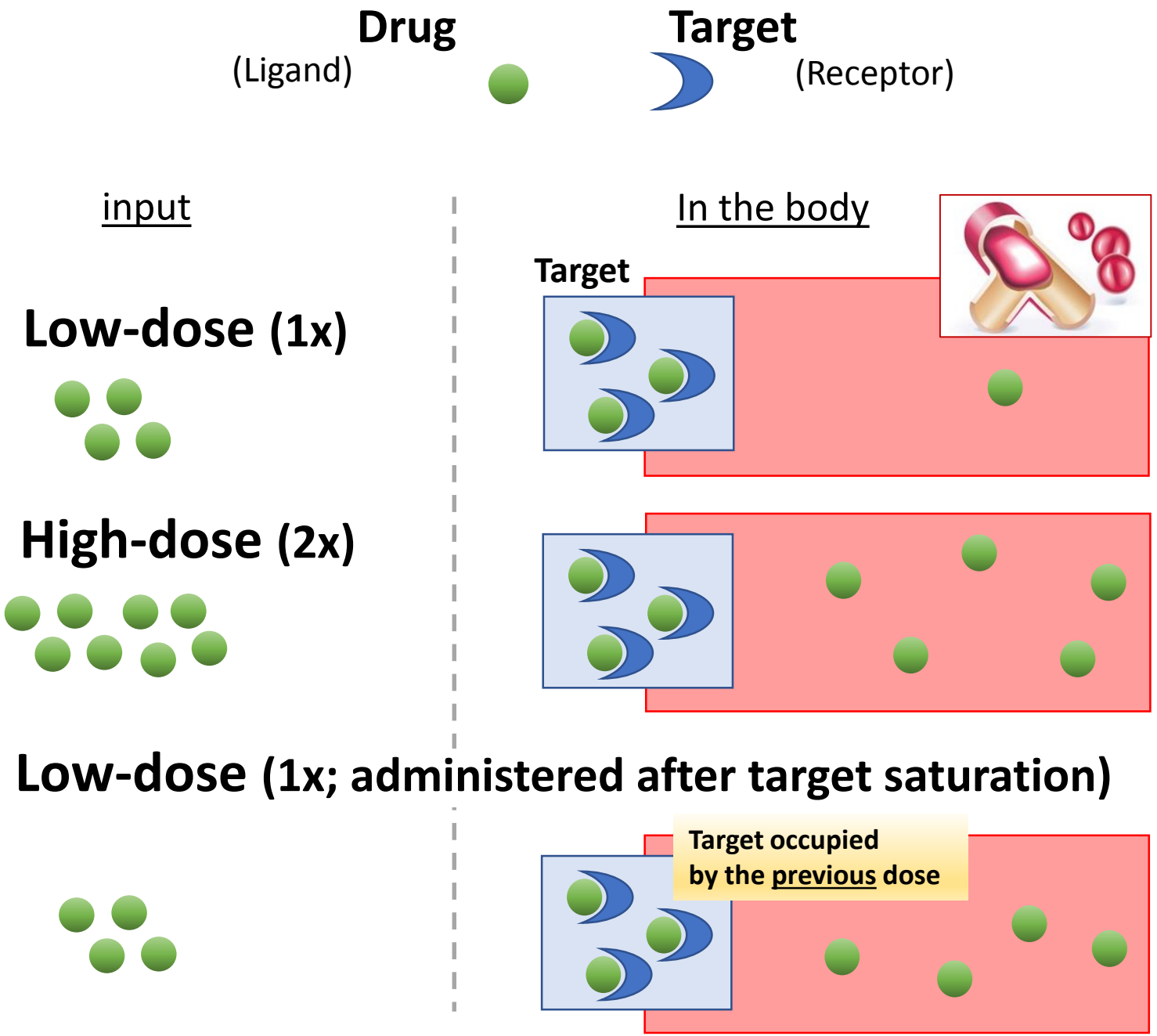
- ✓Introduced by Dr. Gerhard Levy (1994)
- ✓Type of **nonlinear PK**
- ✓**When drugs bind to a target with high affinity and to a significant extent (relative to dose),** part of the initial dose is rapidly acquired by the target sites and only then the drug will distribute to other tissues.

Pharmacologic target-mediated drug disposition

Gerhard Levy, PharmD *Amherst, N.Y.*

(Clin Pharmacol Ther, 1994)

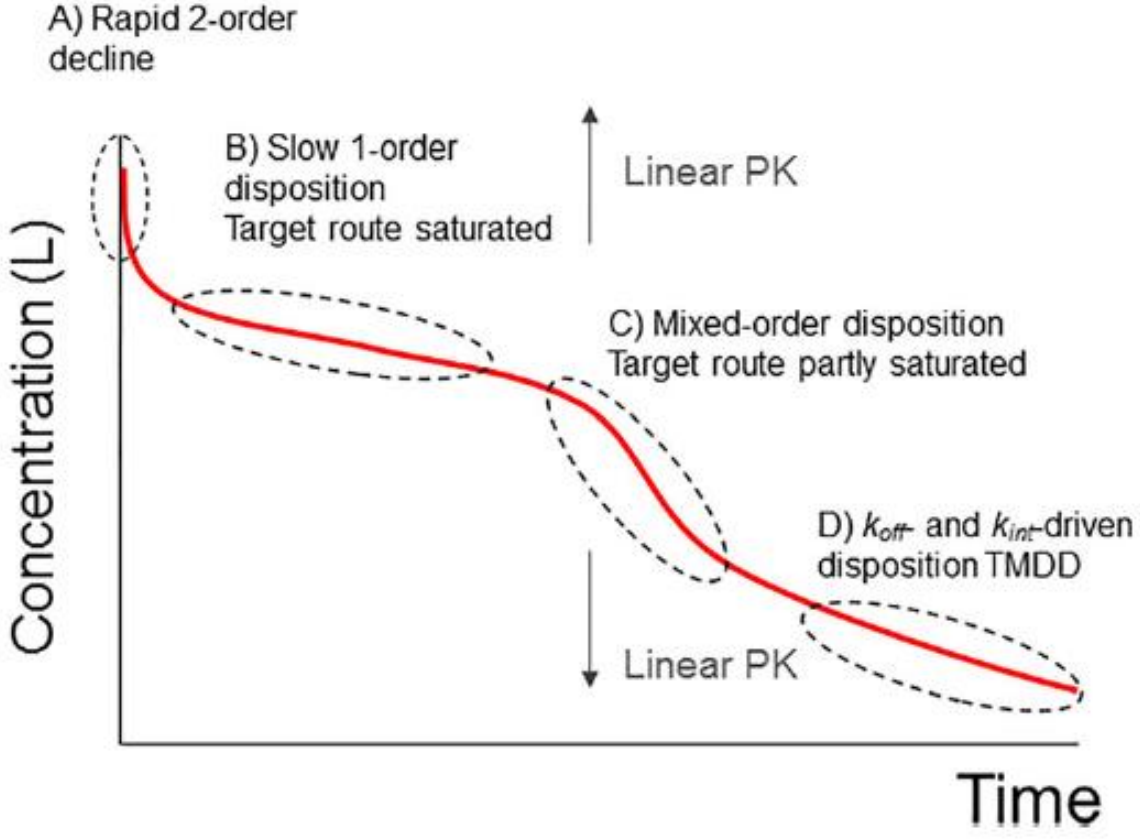
Gerhard Levy, PharmD
(1928-2017)



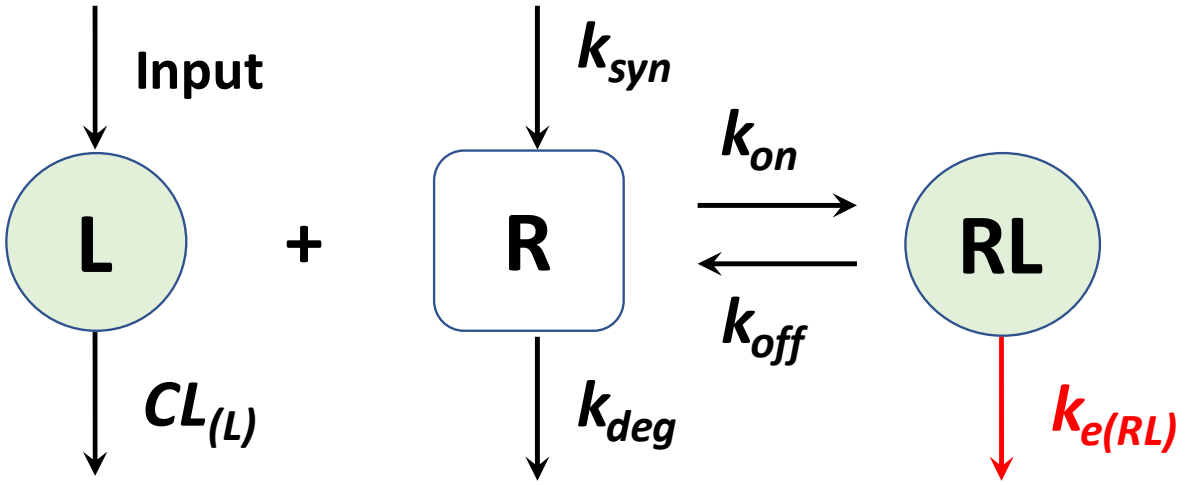
- Saturable (low capacity) binding with high affinity & to a **significant extent**
- Slow turnover
- Formation of Drug-Target complex as a clearance mechanism

"Target binding can impact PK"

TMDD frequently associated with biologics



(Gabrielsson & Peletier, 2017; PMID 28144911)



But also with small-molecule drugs (more cases recognized lately)

Impact of target interactions on small-molecule drug disposition: an overlooked area

Robert A. B. van Waterschoot[1], Neil J. Parrott[1], Andrés Olivares-Morales[1], Thierry Lavé[1], Malcolm Rowland[2] and Dennis A. Smith[3]

(Nat Rev Drug Discov. **2018**, PMID 29472637)

Two insightful reviews published in 2018

Importance of target-mediated drug disposition for small molecules

Dennis A. Smith[1], Robert A.B. van Waterschoot[2], Neil J. Parrott[2], Andrés Olivares-Morales[2], Thierry Lavé[2] and Malcolm Rowland[3]

(Drug Discov Today **2018**, PMID 29928850)

Our On-going Questions..

- **Why was this phenomenon overlooked?**
  (Does this phenomenon happen more often now than before?)

- **When & how** does target binding impact the pharmacokinetics of small-molecule drugs?
- What are the **necessary conditions** for small-molecule drugs to display TMDD?
- Any special considerations/precautions during drug development?

Small-Molecule Drugs with TMDD: A Growing List

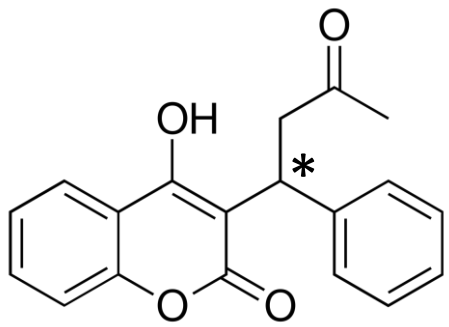
Drug	Target
Warfarin	**vitamin K epoxide reductase (VKORC1)**
Imirestat, Ranirestat	aldose reductase (AR)
Enalaprilat, Perindoprilat, Cilazaprilat	angiotensin-converting enzyme
Selegiline	monoamine oxidase type B
Linagliptin, AMG222	dipeptidyl peptidase-4 (DPP-4)
HSP90 inhibitors (Luminespib)	HSP90
Bosentan	endothelin receptor
Finasteride	steroid 5α-reductase
ABT-384	11β-hydroxysteroid dehydrogenase type 1
PF-04457845	fatty acid amide hydrolase-1 (FAAH1)
Topiramate	Carbonic anhydrase
Bortezomib	proteasome

Earliest example of small-molecule drugs with TMDD

High-affinity, high-potency binding to VKORC1 (K_d in nM ranges)

Warfarin: From rat poison to life-saving medicine

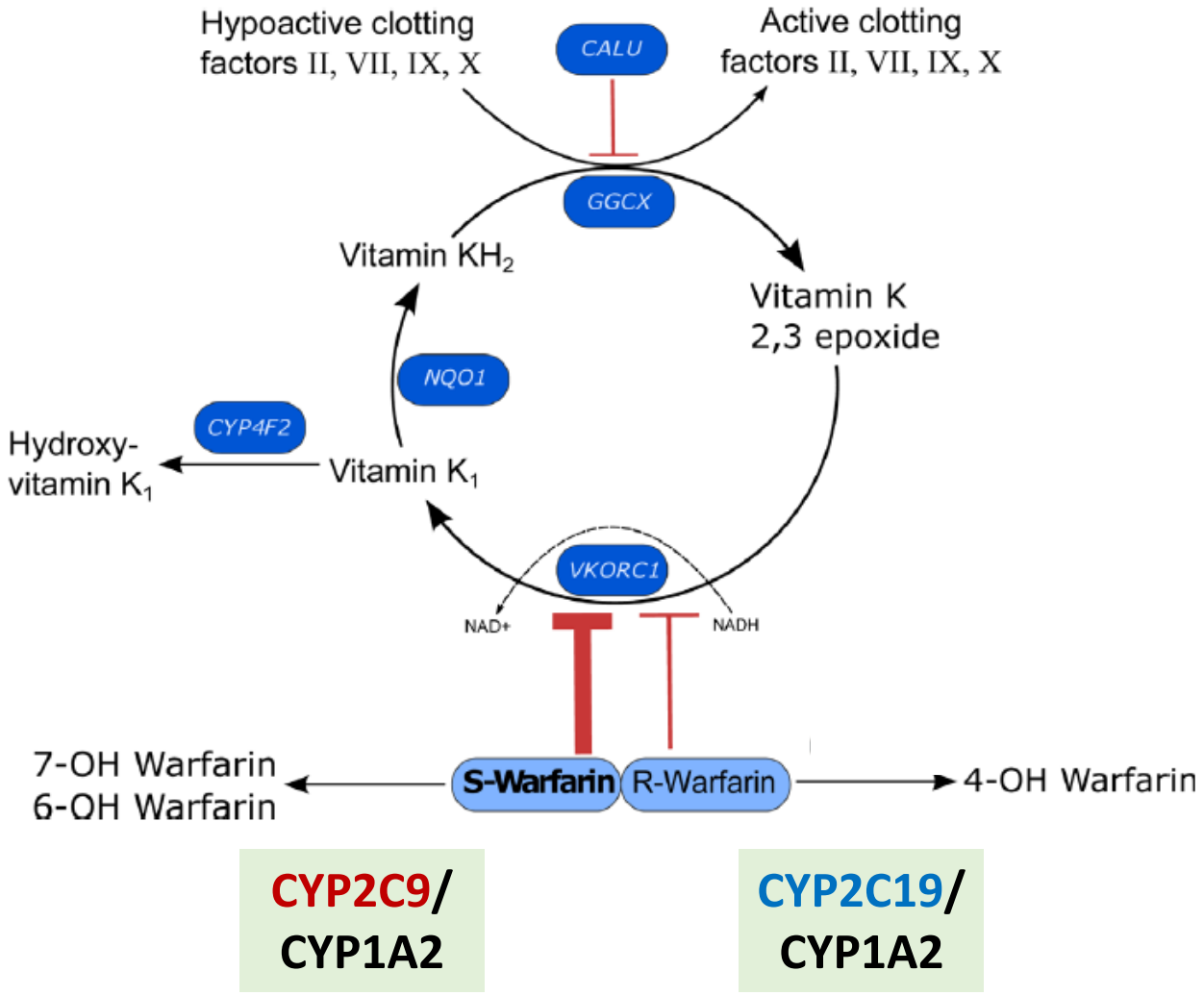
- **1920's**: hemorrhagic disease in cows fed with sweet clover (later **dicoumarol** found as the causal agent by Dr. Link)
- **1948:** potent coumarin derivative warfarin (**W**isconsin **A**lumni **R**esearch **F**oundation + Coum**arin**) proposed as a rodenticide
- **1954**: warfarin (trade name Coumadin) approved as an oral anticoagulant for human use
- vitamin K antagonist; it inhibits **v**
  (**VKORC1**), preventing the forma
  coagulation factors II, VII, IX, & X



High-affinity, high-potency target binding (K_d in nM ranges)

Warfarin therapy: Challenges

- Large inter- & intra-individual variabilities (at both PK & PD levels)
- Narrow therapeutic range

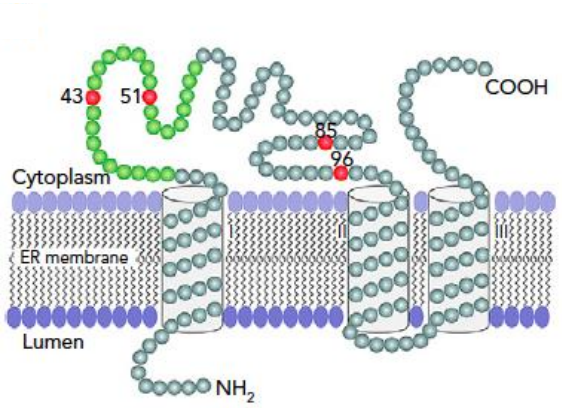


(Kaye et al. 2017; Bi et al. 2018)

Warfarin & VKOR: Continuing story..

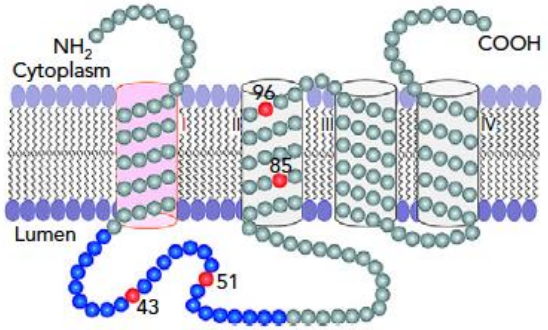
VKOR: 3-transmembrane (TM) or 4-TM domains? Still controversial.

3-TM model



or

4-TM model



● Cysteine

Wu et al. Blood. 2018;132(6):647-657

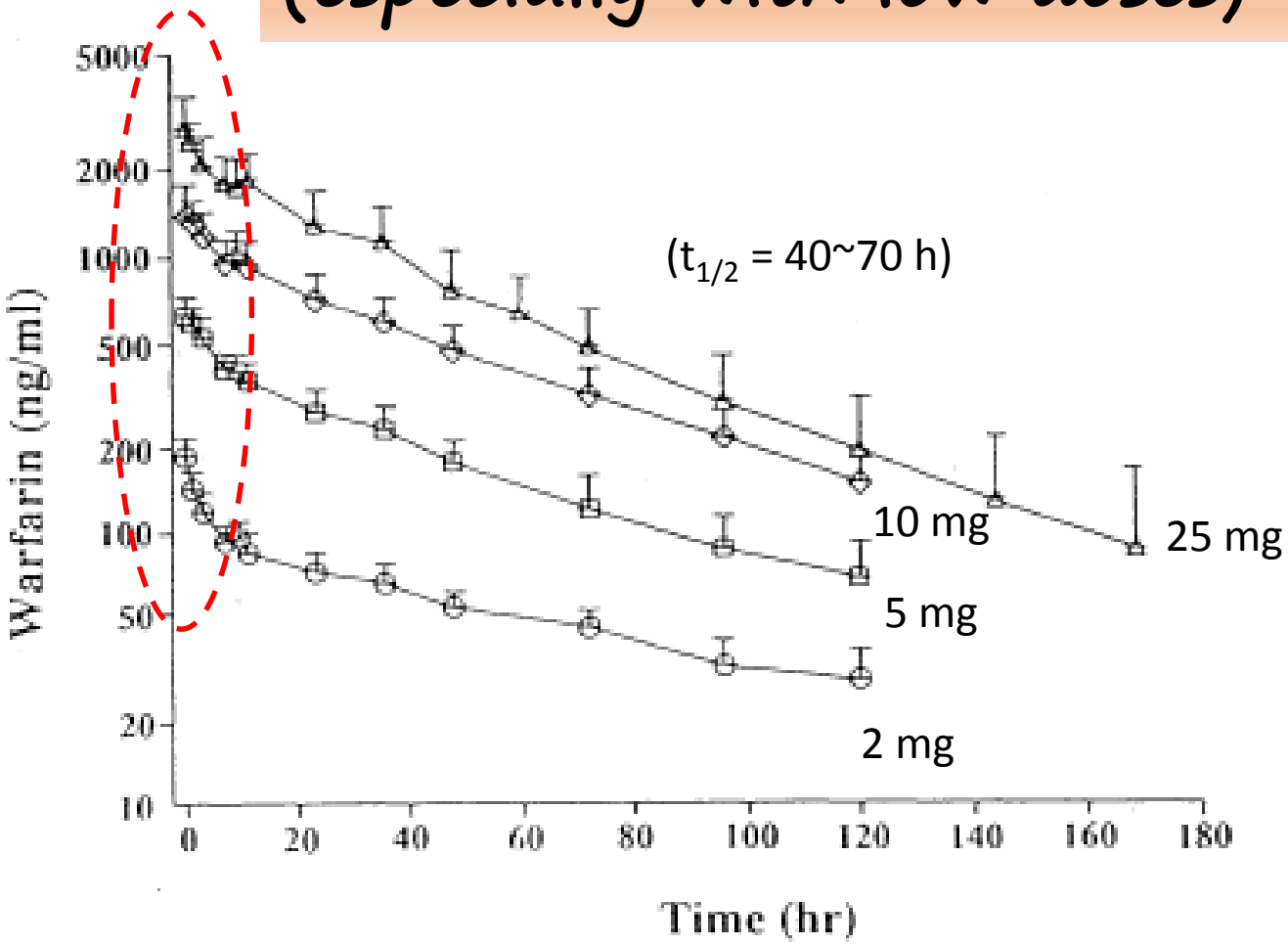
- Binding of warfarin to VKOR is impacted by the redox status and disulfide bridges of Cys residues (possibly accounting for **varying potency depending on assay platforms**)
- Binding of warfarin to VKOR: **high-affinity, high-potency** (K_D of ~30-60 nM from a radiolabeled binding study using rat liver microsomes; PMID 2706010)

Warfarin: Nonlinear PK in Humans

Oral administration of
racemic warfarin
to healthy volunteers

R-/S-warfarin
not separated

Rapid initial distribution (especially with low doses)



King et al. (1995)

Pharmaceutical Research, Vol. 12, No. 12, 1995

Warfarin: Nonlinear PK in Humans

King et al (1995)

Table I. Pharmacokinetic Parameters of Warfarin After Administration of Single Oral Doses of Coumadin Tablets in Healthy Volunteers[a]

Parameter	2-mg (n = 19)[c]	5-mg (n = 19)[c]	10-mg (n = 18)[c]	25-mg[b] (n = 12)[c]
C_{max} (μg/ml)	0.19 ± 0.03	0.65 ± 0.08	1.4 ± 0.3	3.0 ± 0.8
t_{max} (hr)[d]	1.0 (1.0, 1.0)	1.0 (1.0, 4.0)	1.0 (1.0, 4.0)	1.0 (1.0, 2.0)
$AUC_{0-\infty}$ (μg · hr/ml)	10 ± 2	29 ± 6	62 ± 16	120 ± 30
$\lambda_n \times 100$ (hr^{-1})	0.98 ± 0.19[e]	1.5 ± 0.2	1.7 ± 0.3	1.8 ± 0.4
$t_{1/2}$ (hr)[f]	71 ± 14[e]	47 ± 8	41 ± 7	38 ± 8
CL/F (ml/min)	3.3 ± 0.7	3.1 ± 0.6	2.8 ± 0.7	3.7 ± 1.3
V/F (l)	21 ± 4[e]	12 ± 2	10 ± 2	12 ± 4

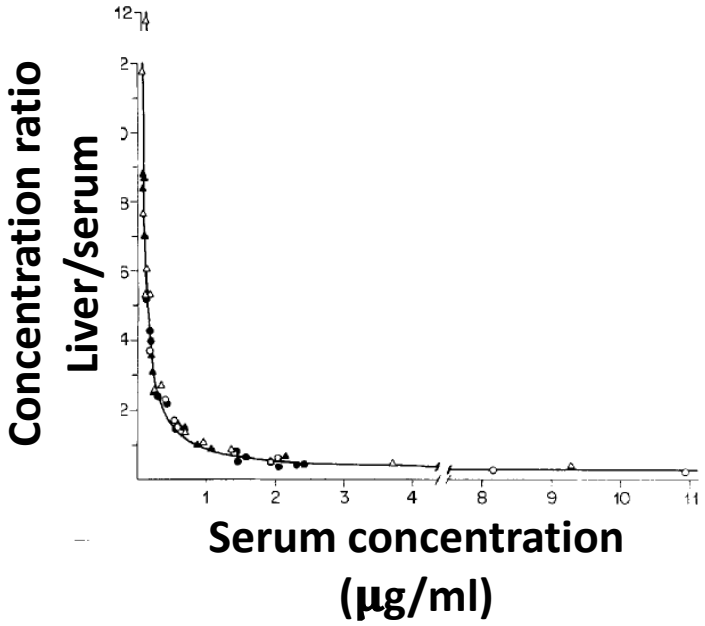
[a] All data are expressed as mean ± SD.

With increasing warfarin doses,
CL: no change
V_d: ↓

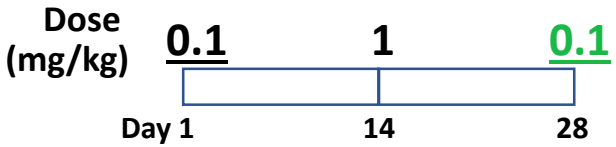
Mechanism(s)??

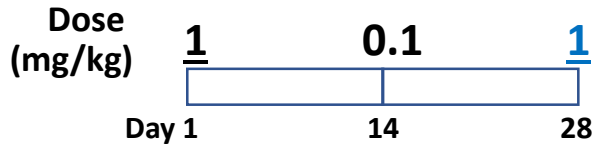
Saturable target binding: Sequential dosing in rats

Takada & Levy, 1980, PMID: 7354452

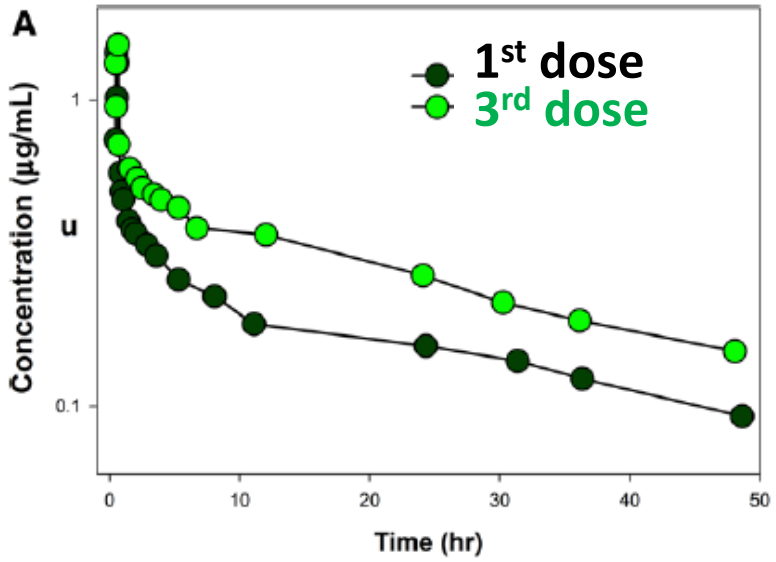


Same **rats** received 3 doses at 2-week intervals

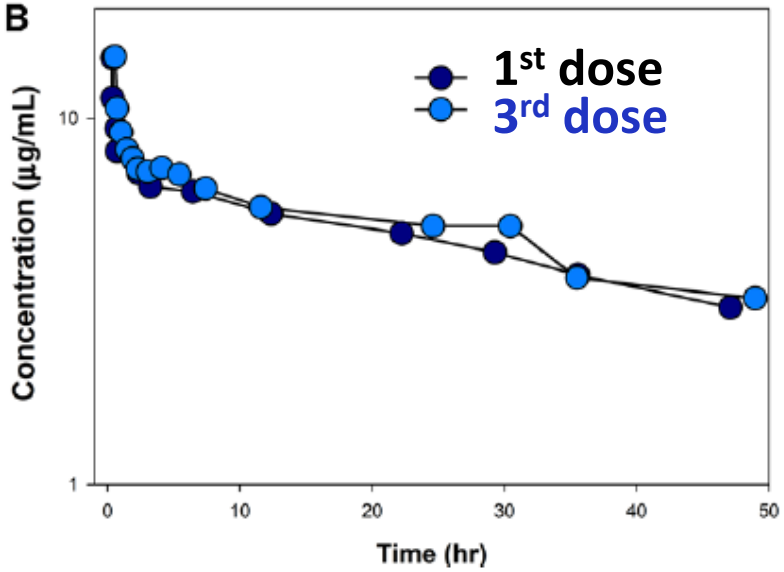




0.1 mg/kg

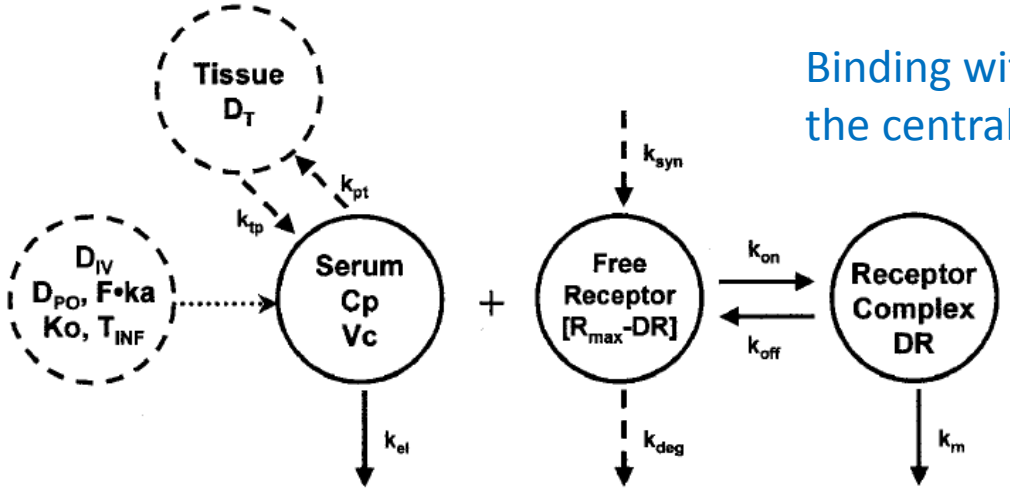


1 mg/kg



1 mg/kg warfarin dose: sufficiently high to saturate the high-affinity binding to VKOR

Warfarin: Compartmental PK Model (humans; Levy et al. 2003)



Binding with the drug in the central compartment

General Pharmacokinetic Model for Drugs Exhibiting Target-Mediated Drug Disposition

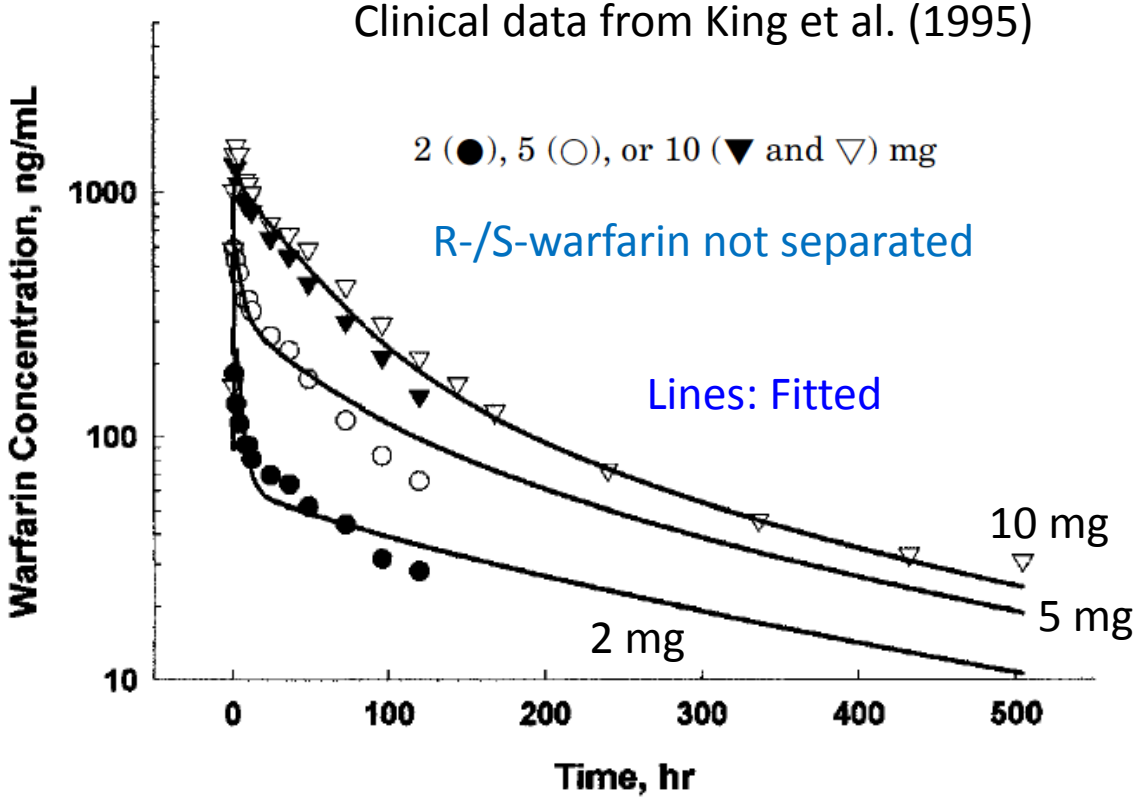
Donald E. Mager[1] and William J. Jusko[1,2]

$$\frac{dCp}{dt} = \mathrm{In}(t) - (k_{el} + k_{pt}) \cdot Cp + k_{tp} \cdot \frac{D_T}{Vc} - k_{on} \cdot (R_{max} - \mathrm{DR}) \cdot Cp + k_{off} \cdot \mathrm{DR}$$

$$\frac{dD_T}{dt} = k_{pt} \cdot Cp \cdot Vc - k_{tp} \cdot D_T$$

$$\frac{d\mathrm{DR}}{dt} = k_{on} \cdot (R_{max} - \mathrm{DR}) \cdot Cp - (k_{off} + k_m) \cdot \mathrm{DR}$$

Clinical data from King et al. (1995)



R-/S-warfarin not separated

Lines: Fitted

Levy et al. J Pharm Sci. 2003; PMID 12712418

Warfarin: Compartmental PK Model (humans; Levy et al. 2003)

(2003)

Table 3. Target-Mediated Warfarin Disposition Modeling Parameters in Man

Parameter[a]	Racemic Warfarin[b]
k_e, h^{-1}	0.0193 (11)
k_{on}, $\mu M^{-1} h^{-1}$	0.126 (20)
k_{off}, h^{-1}	0.0405 (22)
R_{max}, $\mu moles \cdot kg^{-1}$	0.167 (14)
V_c, $L \cdot kg^{-1}$	0.0745 (11)
k_a, h^{-1}	1.19 (19)

[a]Model parameters (Case A of Reference[2]) are: k_e, first order elimination rate constant; k_{on}, second order rate constant for formation of drug-target complex; k_{off}, first order rate constant for dissociation of drug-target complex; R_{max}, total density of target protein; V_c, volume of central compartment; k_a, first order absorption rate constant.

[b]Coefficient of variation in parentheses.

$K_d = k_{off}/k_{on} = 0.321$ µM

for unbound warfarin, ~ 4.1 nM

(assuming the free fraction in plasma 1.3%)

Dissociation $t_{1/2} = 0.693/0.0405 = 17.1$ h

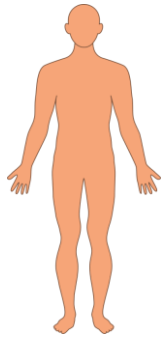
$R_{max} = 0.167$ µmole

-> 10.2 µmole for 60 kg

warfarin therapeutic dose		
mg		micromole
2		6.49
5		16.23
10		32.47

Prediction of human PK: Microdosing approach

Dose extrapolation in humans



Microdose

Therapeutic dose

"Human is the best model for human"

	Approach 1	Approach 2
	Microdosing	
Dose Definition	≤ 1/100th NOAEL and ≤ 1/100th of pharmacologically active dose (scaled on mg/kg for IV and mg/m² for oral)	Same as approach 1
Cumulative Dose	100 µg	500 µg
Limit per Dose	100 µg	100 µg
Maximal Daily Dose	100 µg	100 µg
Number / duration of Dosing	**1** (could be divided to multiple doses with a total of 100 µg)	**5**
Washout	No washout	6 or more half-lives between doses

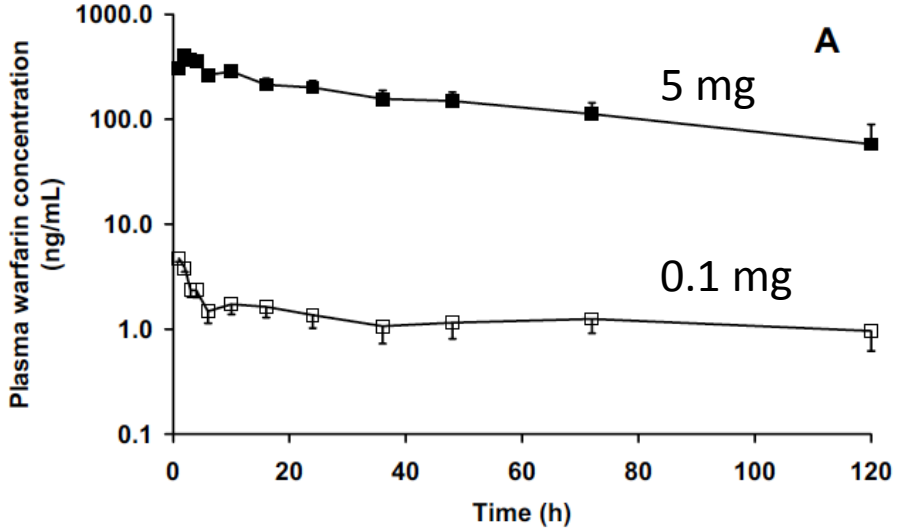
Key questions for dose extrapolation:

- Is there **non-linearity in the microdose *vs* therapeutic-level exposure range**?
- If so, by what mechanisms? (saturable metabolism, transport and/or target binding)?

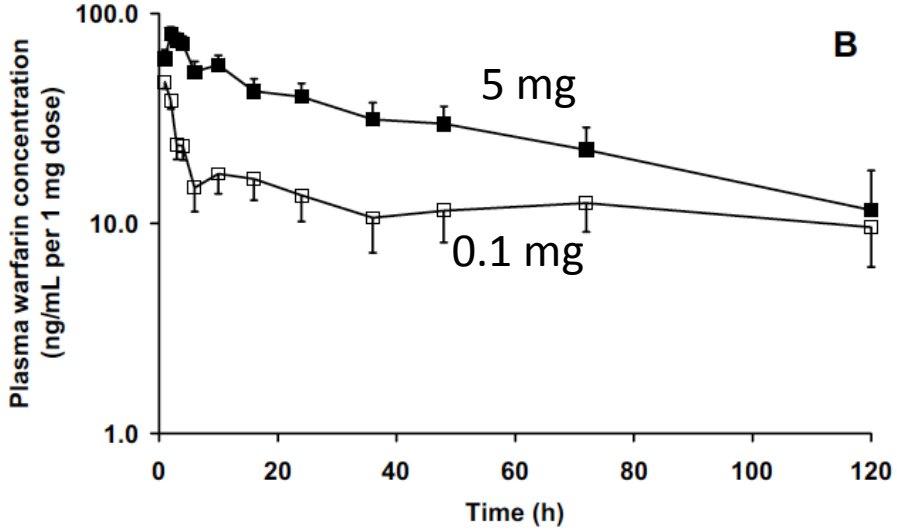
Warfarin: Microdose vs Full Dose (Lappin et al. 2006)

Warfarin dose (mg)	CL (L/h)	**V** (L)
0.1	0.17 (54.8)	**67.3** (41.4)
5	0.26 (48.1)	**17.9** (19.9)

Microdosing (0.1 mg) of warfarin did NOT predict the PK at the therapeutic dose (5 mg)



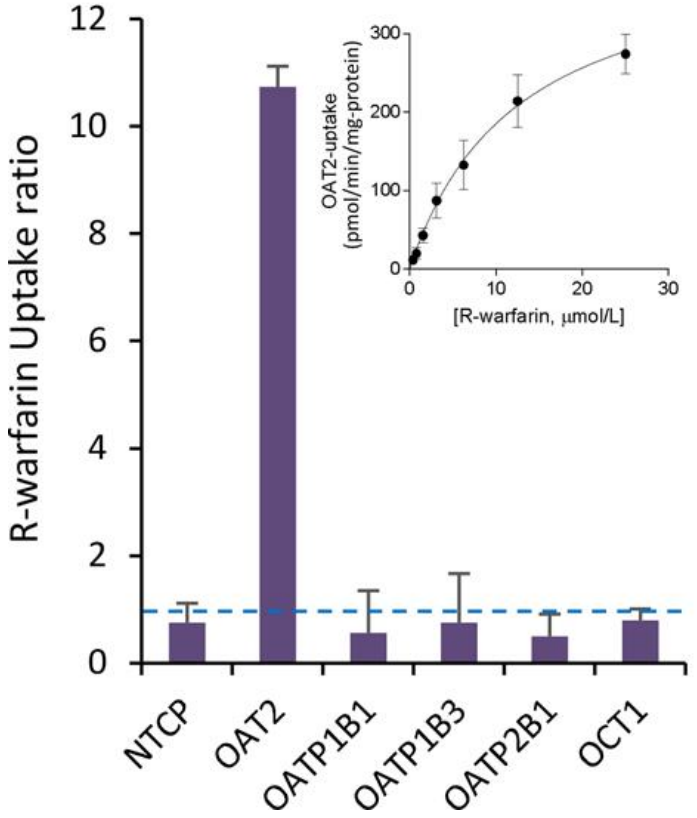
Dose-normalized

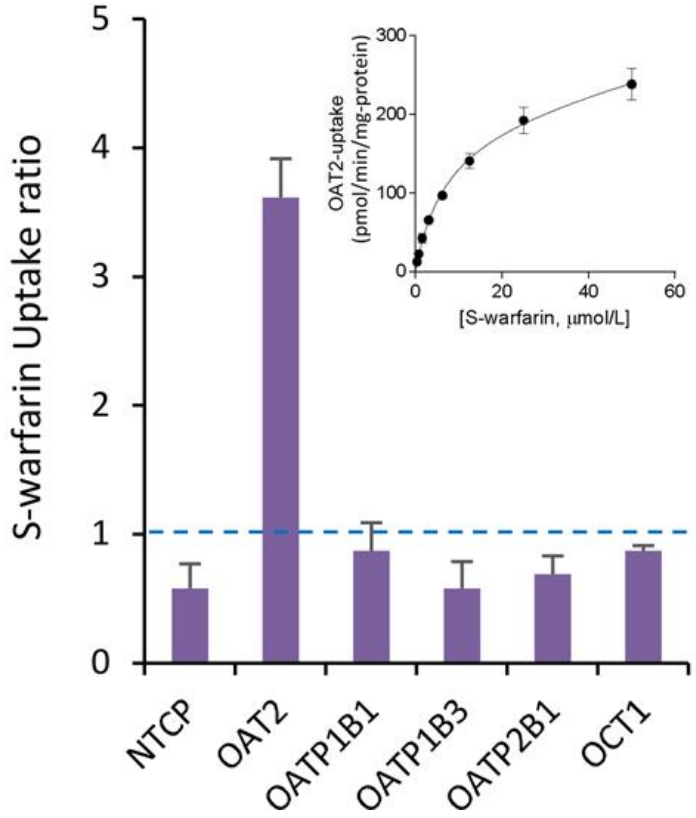


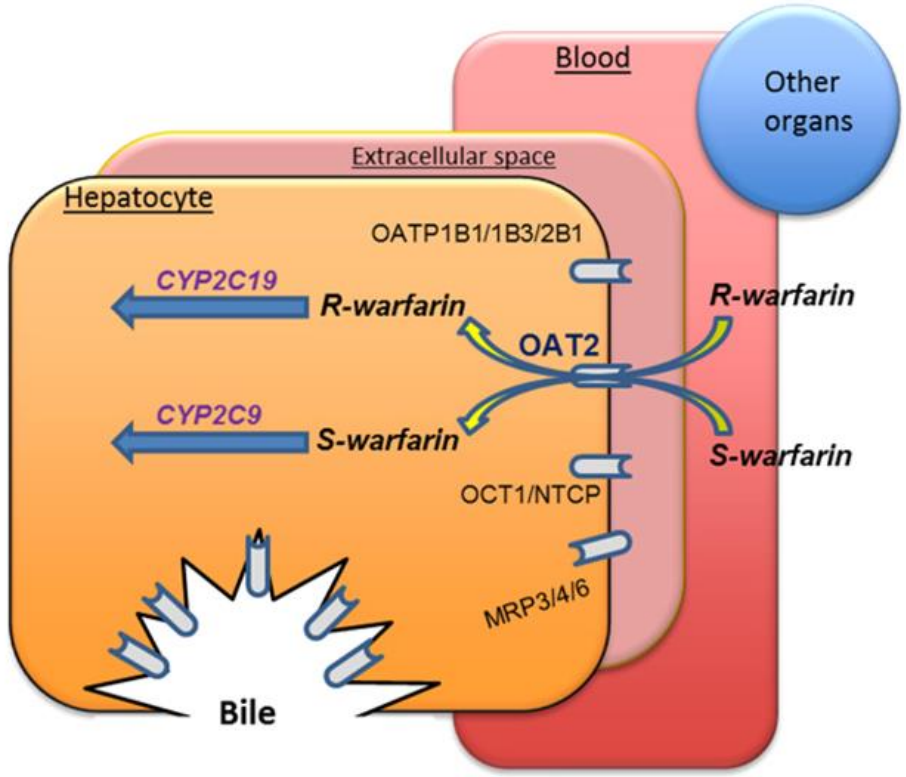
(Lappin et al. Clin Pharmacol Ther. 2006 PMID 16952487)

Warfarin: PBPK Model with CYP/OAT2 Interplay (Bi et al. 2018)

Interpatient variability in S-warfarin clearance is only partially (~20-30%) explained by CYP2C9 genotypes. -> ***Involvement of hepatic transporters??***







(Bi et al. 2018; PMID: 29433307)

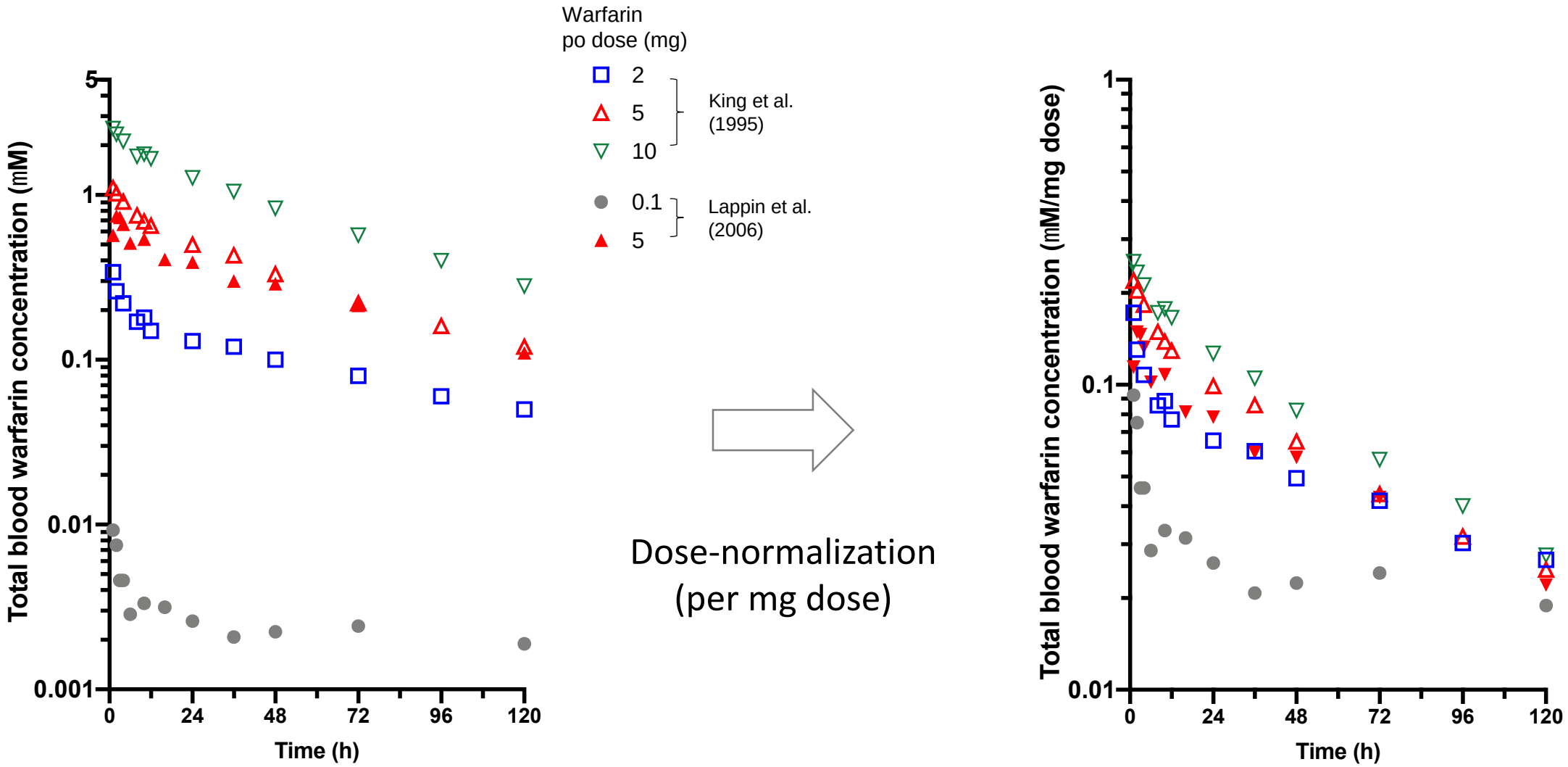
Goals

1. **Establish a PBPK model for warfarin incorporating our current molecular understanding of warfarin disposition processes;**

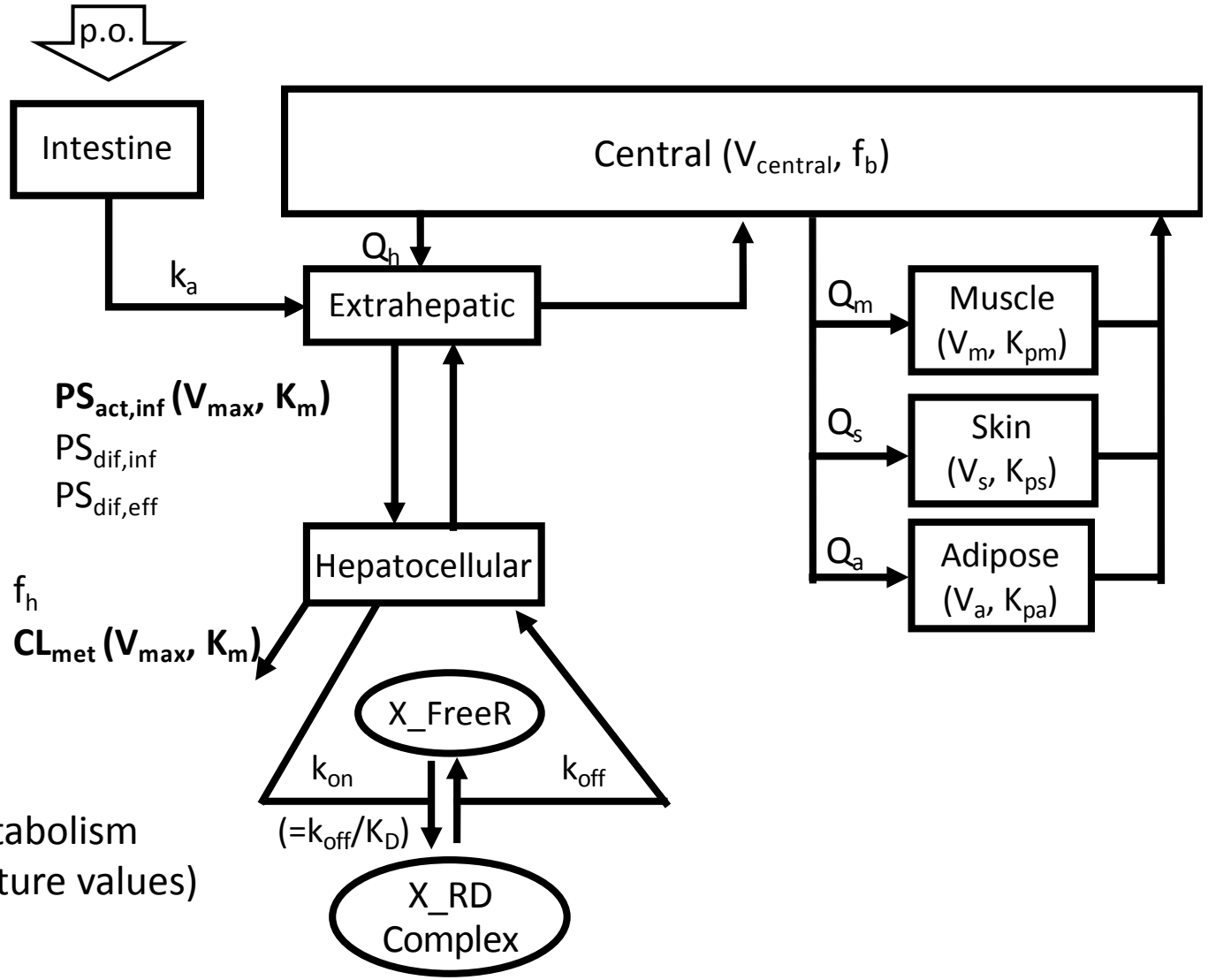
   - **Saturable target binding in the liver**
   - OAT2/CYP interplay (Bi et al. 2018)

2. Identify optimal conditions (range of doses or concentrations) for reliable estimation of target binding parameters (K_D, Rc occupancy) based on blood drug concentration profiles only

Warfarin Observed PK Data



Warfarin PBPK Model: Structure



for both hepatic uptake & metabolism
- Km (fixed based on the literature values)
- Vmax (optimized)

d**X_Abs_po0_1**dt= - *ka* * **X_Abs_po0_1**

d**C_Central_po0_1**dt=1 / *Vcentral* * (*Qh* * (**C_HepEx_po0_1** - **C_Central_po0_1**) - *Qm* * (**C_Central_po0_1** - **C_Muscle_po0_1** / *Kpm*) - *Qs* * (**C_Central_po0_1** - **C_Skin_po0_1** / *Kps*) - *Qa* * (**C_Central_po0_1** - **C_Adipose_po0_1** / *Kpa*))

d**C_HepEx_po0_1**dt=1 / *Vhe* * (*ka* * **X_Abs_po0_1** + *Qh* * (**C_Central_po0_1** - **C_HepEx_po0_1**) - (*Vm*ax_act_inf / (*Km_act_inf* + *fb* * **C_HepEx_po0_1**) * *fb* * **C_HepEx_po0_1** + (*Vm*ax_act_inf / *Km_act_inf*) * *R_dif* * *fb* * **C_HepEx_po0_1** - (*Vm*ax_act_inf / *Km_act_inf*) * *R_dif* / 0.243 * *fh* * **C_Hep_po0_1**))

d**C_Hep_po0_1**dt=1 / *Vh* * (*Vm*ax_act_inf / (*Km_act_inf* + *fb* * **C_HepEx_po0_1**) * *fb* * **C_HepEx_po0_1** + (*Vm*ax_act_inf / *Km_act_inf*) * *R_dif* * *fb* * **C_HepEx_po0_1** - (*Vm*ax_act_inf / *Km_act_inf*) * *R_dif* / 0.243 * *fh* * **C_Hep_po0_1** - (*Vm*ax_met / (*Km_met* + *fh* * **C_Hep_po0_1**) * *fh* * **C_Hep_po0_1**) - *k_off* / *Kd* * *fh* * **C_Hep_po0_1** * **X_FreeR_po0_1** + *k_off* * **X_RDcomplex_po0_1**)

Target binding inside hepatocytes

d**X_FreeR_po0_1**dt=*k_off* * **X_RDcomplex_po0_1** - *k_off* / *Kd* * *fh* * **C_Hep_po0_1** * **X_FreeR_po0_1**

d**X_RDcomplex_po0_1**dt=*k_off* / *Kd* * *fh* * **C_Hep_po0_1** * **X_FreeR_po0_1** - *k_off* * **X_RDcomplex_po0_1**

d**C_Muscle_po0_1**dt=1 / *Vm* * *Qm* * (**C_Central_po0_1** - **C_Muscle_po0_1** / *Kpm*)
d**C_Skin_po0_1**dt=1 / *Vs* * *Qs* * (**C_Central_po0_1** - **C_Skin_po0_1** / *Kps*)
d**C_Adipose_po0_1**dt=1 / *Va* * *Qa* * (**C_Central_po0_1** - **C_Adipose_po0_1** / *Kpa*)

d**R_occupancy_po0_1**dt=(*k_off* / *Kd* * *fh* * **C_Hep_po0_1** * **X_FreeR_po0_1** - *k_off* * **X_RDcomplex_po0_1**) / *X_TotalR*

Rc occupancy calculated by [RDcomplex_po0_1] divided by X_TotalR

Cluster Newton Method (CNM)

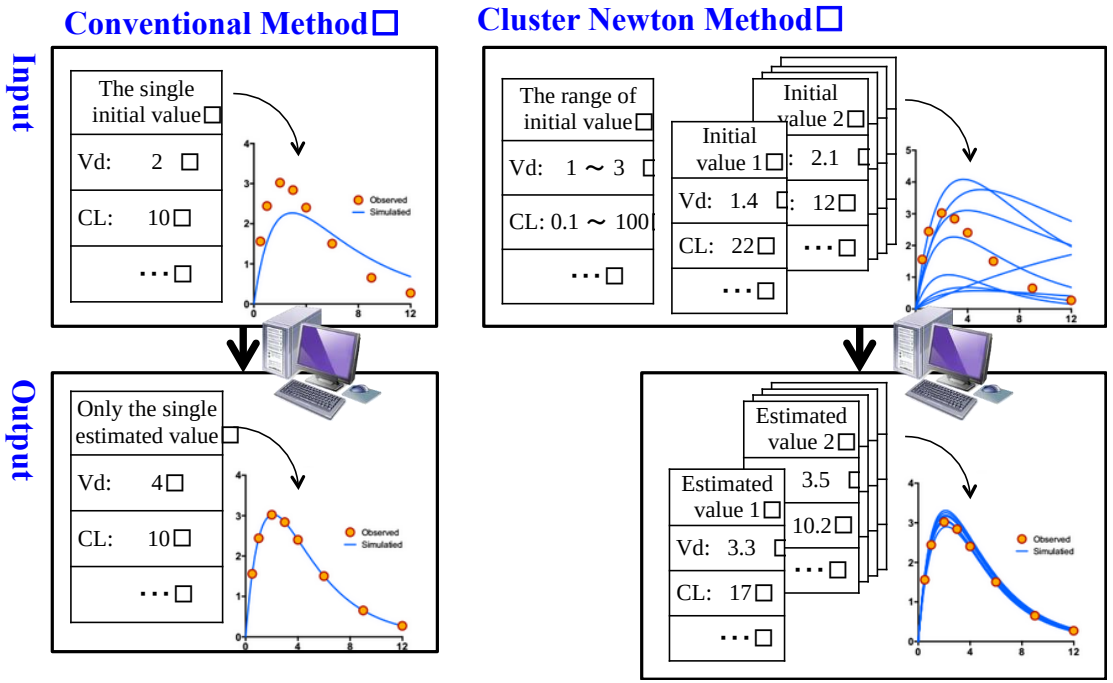
For finding multiple solutions of underdetermined inverse problems.

Conventional method (e.g. Gauss-Newton method)

- Requires appropriate initial value for parameters.
- Obtains only a single set of optimized parameters.

Cluster Newton method

- Requires only setting wide ranges for initial values of parameters.
- Obtains multiple sets of optimized parameters.
- Can estimate many unknown parameters.

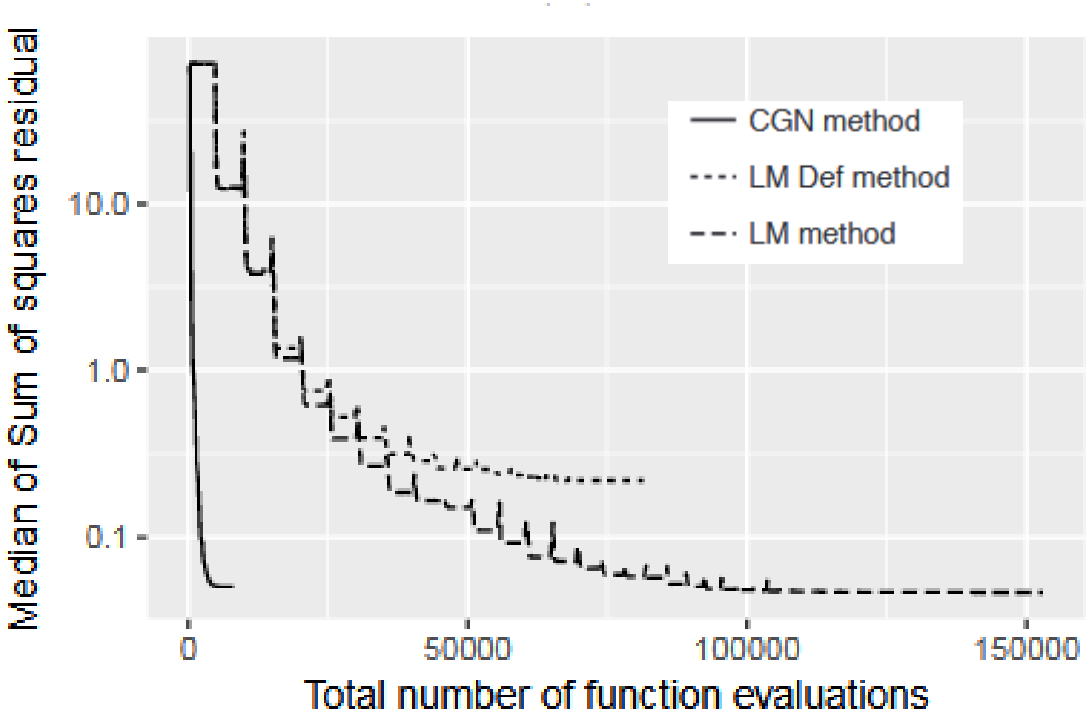


Aoki Y et al. SIAM J Sci Comp 36:B14-B44 (2014).

Slide prepared by K. Toshimoto (Riken, Sugiyama Laboratory)

Cluster Gauss-Newton Method (CGNM)

- CGNM allows for optimization of multiple sets of parameters which minimize the objective function such as the sum of squares residual.
  - Update the next values for each set using weighted linear regression (CNM-like regression) instead of Jacobian (Gauss-Newton Method).



Preprint version is uploaded by arXiv.org.
https://arxiv.org/abs/1808.06714

Cluster Gauss-Newton method for sampling multiple solutions of nonlinear least squares problems - with applications to pharmacokinetic models

Yasunori Aoki, Ken Hayami, Kota Toshimoto, Yuichi Sugiyama

(Submitted on 20 Aug 2018 (v1), last revised 4 Oct 2018 (this version, v2))

Slide prepared by K. Toshimoto (Riken, Sugiyama Laboratory)

Warfarin PBPK model parameters

Fixed parameters

Parameter	Unit	Value	Ref
$K_{m(met)}$	µmole/L	10	Shaik et al., 2016
$K_{m(act,influx)}$	µmole/L	8.9	Bi et al., 2018
K_{pa}		0.883	Rodgers & Rowland, 2006
K_{pm}		0.115	
K_{ps}		0.477	
Q_h	L/h	96.9	Davies & Morris, 1993
Q_a	L/h	17.4	
Q_m	L/h	50.1	
Q_s	L/h	20.1	
$V_{central}$	L	**4.47**	Levy et al., 2003
V_{he}	L	0.521	Kawai et al., 1998
V_h	L	1.36	Davies & Morris, 1993
V_a	L	11.1	
V_m	L	33.4	
V_s	L	8.69	
f_b		**0.022**	(0.013/0.59) Bi et al., 2018
f_h		**0.69**	Bi et al., 2018

Using CGNM
1,000 parameter sets,
100 iterations/run

Fitted parameters (Initial ranges were set 1/100 – 100 fold of the base values from the literature)

Parameter	Unit	Initial min	Initial max
K_d	µM	0.0032	32
V_{max_met}	µmol/h	3.5	35000
V_{max_uptake}	µmol/h	26	260000
k_{off}	/h	0.000405	4.05
k_a	/h	0.1	6
Total_R	µmol	0.1	1000

Results: SSR

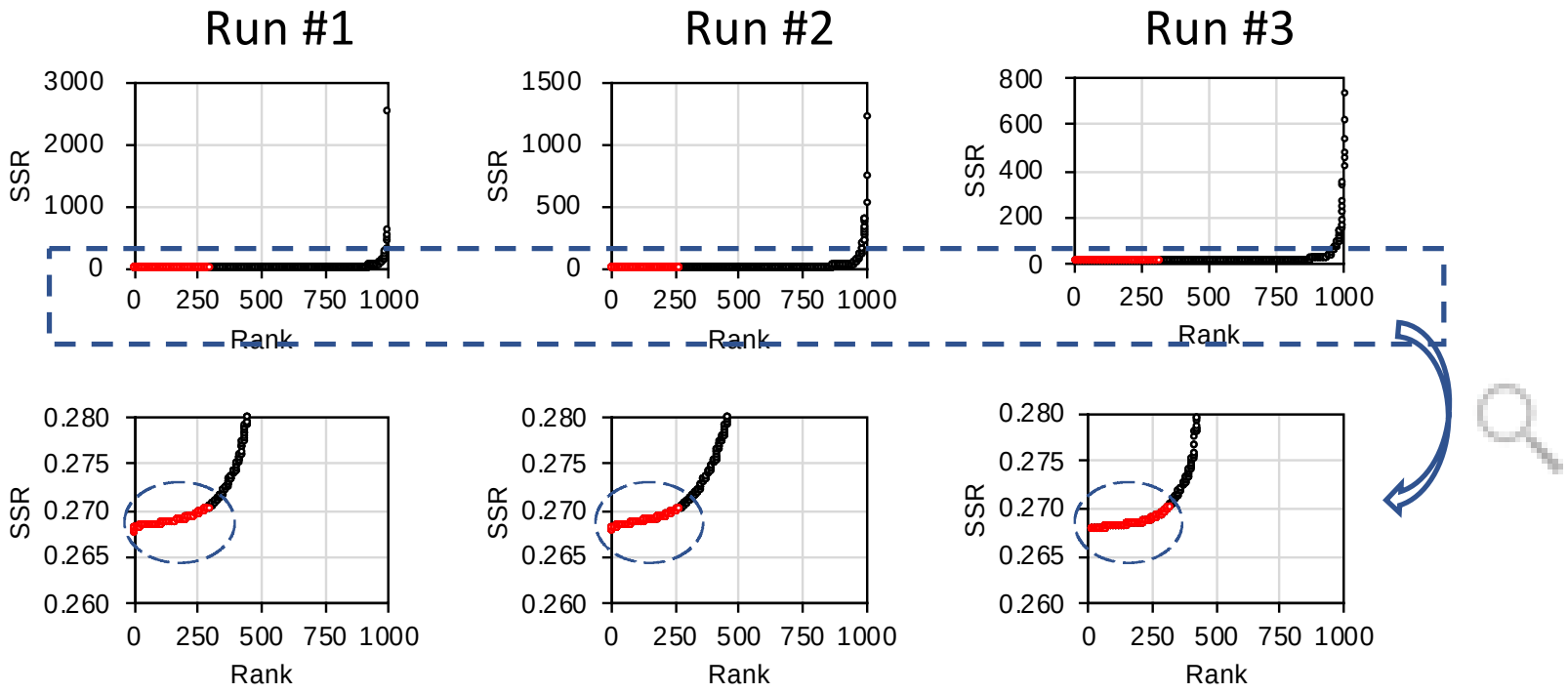
CGNM run
1,000 parameter sets via
100 iterations per run

SSR values plotted in the ascending order

SSR cut-off =0.27

Parameter sets with SSR values less than cut-off values (marked in red) were used for subsequent analysis

Repeated CGNM runs with different initial clusters



	N=295 sets		N= 269 sets		N= 309 sets	
	rank		rank		rank	
	1	188	1	269	1	309
SSR	0.2673	0.2670	0.2677	0.2700	0.2677	0.2700

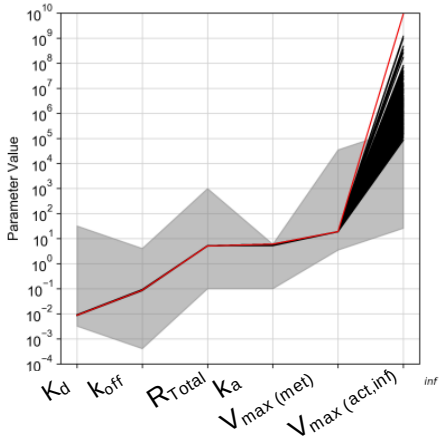
Results

Repeated CGNM runs
with different initial clusters

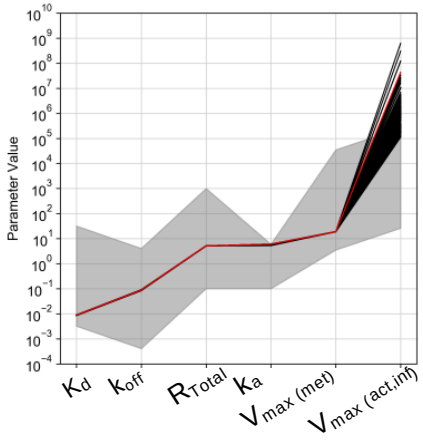
King et al.			Lappin et al.	
2	5	10	0.1	5
✓	✓	✓	✓	✓

Parameter distribution

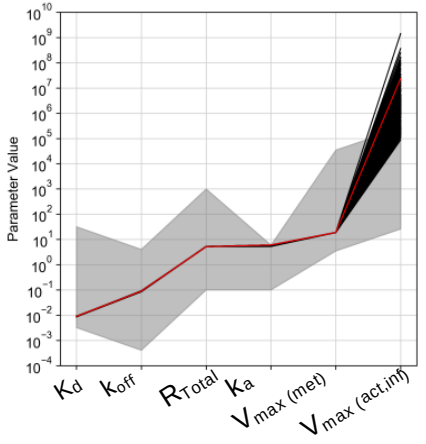
Run #1



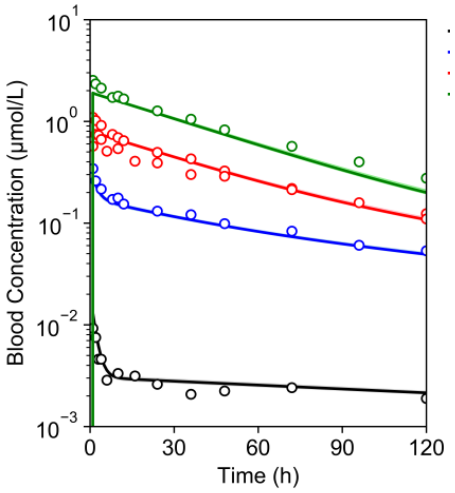
Run #2

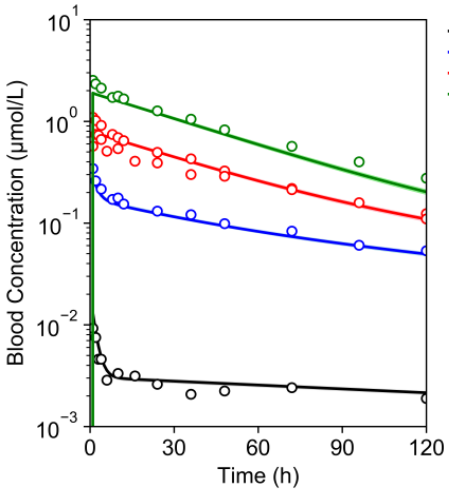


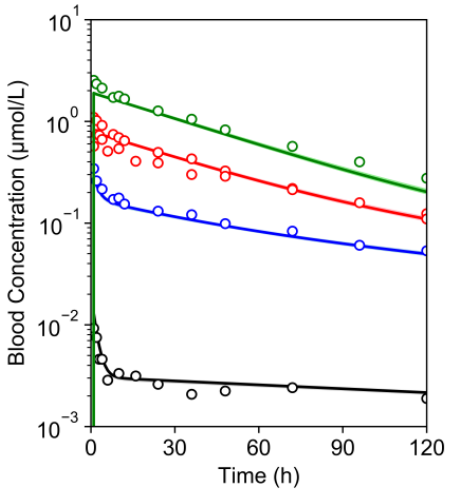
Run #3



Blood PK profiles







Warfarin
po dose (mg)

- 0.1
- 2
- 5
- 10

All the parameters sets below SSR cutoff (0.27) yielded simulated blood PK profiles in good agreement with the observed data.

(in narrow ranges, all lines nearly overlapping)

Results: Optimized Parameters

King et al.			Lappin et al.	
2	5	10	0.1	5
✓	✓	✓	✓	✓

	Run #1 (N= 295 sets)				Run #2 (N= 269 sets)				Run #3 (N= 309 sets)			
	Rank 1	max	min	median	Rank 1	max	min	median	Rank 1	max	min	median
Optimized												
K_d (nM)	8.65	9.10	8.41	8.78	8.75	8.90	8.39	8.77	8.80	9.00	8.44	8.79
k_{off} (/h)	0.087	0.093	0.083	0.088	0.087	0.090	0.083	0.088	0.088	0.092	0.083	0.087
R_{Total} (µmole)	5.06	5.19	4.98	5.12	5.12	5.17	4.99	5.12	5.13	5.27	5.05	5.14
$V_{max(met)}$ (µmole/h)	18.89	19.03	18.51	18.75	18.76	19.07	18.48	18.78	18.74	18.99	18.38	18.67
$V_{max(act,inf)}$ (µmole/h)	9.12×10^{9}	9.12×10^{9}	8.43×10^{4}	1.22×10^{6}	4.34×10^{7}	6.18×10^{8}	1.11×10^{5}	9.25×10^{5}	2.36×10^{7}	1.43×10^{9}	8.72×10^{4}	1.84×10^{6}
k_a (/h)	5.99	6.00	5.18	5.82	5.98	6.00	5.25	5.80	5.97	6.00	5.24	5.88
$CL_{int,all}$ (L/h)*	13.39	13.49	13.12	13.29	13.29	13.52	13.10	13.31	13.29	13.46	13.02	13.23

* Calculated based on the extended clearance concept model

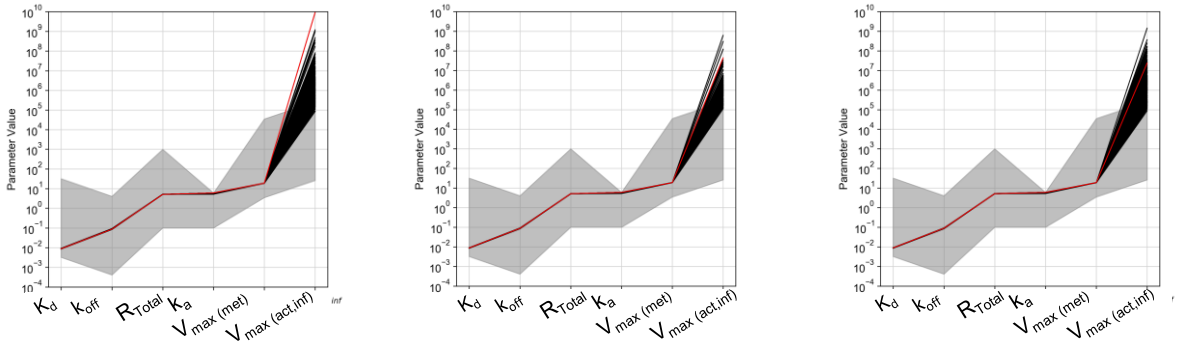
Results

Repeated CGNM runs
with different initial clusters

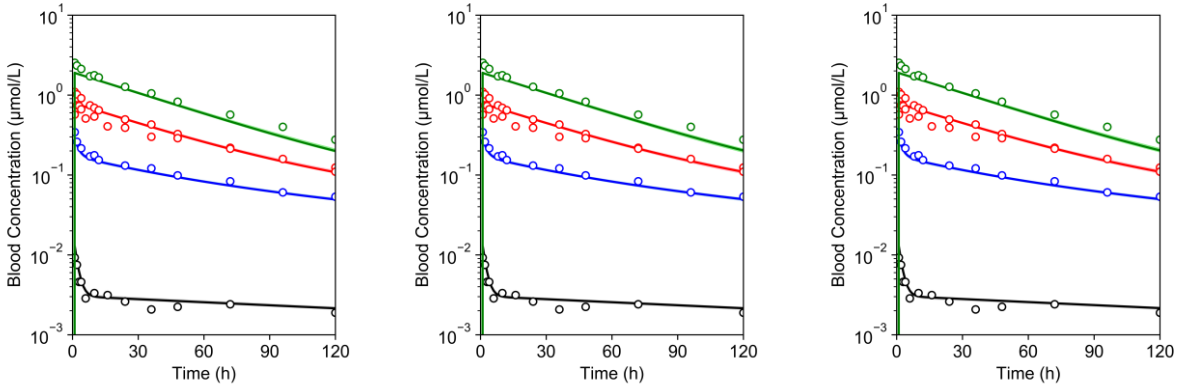
King et al.			Lappin et al.	
2	5	10	0.1	5
✓	✓	✓	✓	✓

Run #1 Run #2 Run #3

Parameter distribution

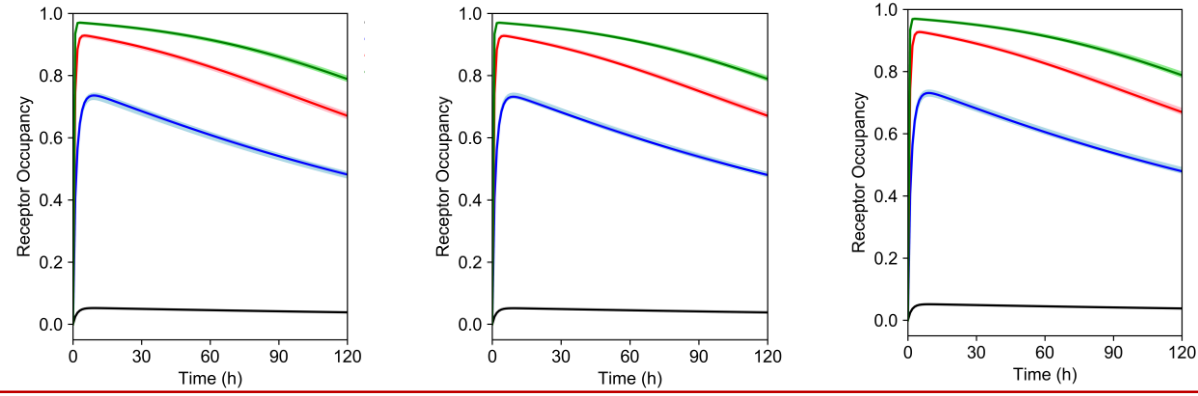


Blood PK profiles



Warfarin
po dose (mg)
0.1
2
5
10

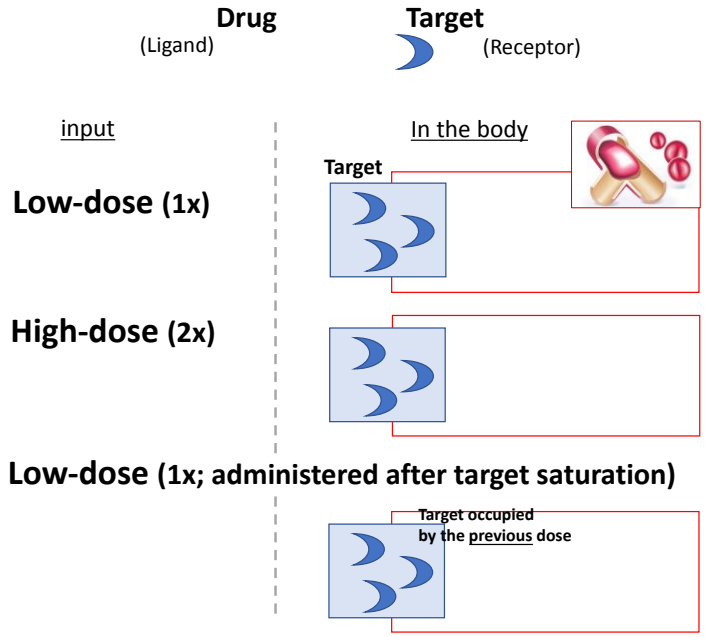
Simulated target occupancy profiles



All the parameters sets below SSR cutoff (0.27) yielded simulated target occupancy profiles in a narrow range.

(all lines nearly overlapping)

Factors of potential importance



- Saturable (low capacity) binding with high affinity & to a **significant extent**
- Slow turnover
- Formation of Drug-Target complex as a clearance mechanism

"Target binding can impact PK"

Types of impact may depend on the location of target binding: ***circulation- vs tissue-based***

- Target location (circulation- or tissue-based)
- Affinity to target, slow dissociation from target (K_d, k_{on}, k_{off})
- Specificity to target (relative to other disposition components; nonspecific binding & non-target-mediated)
- **Target abundance (significant relative to drug dose)**
- Target turnover rate

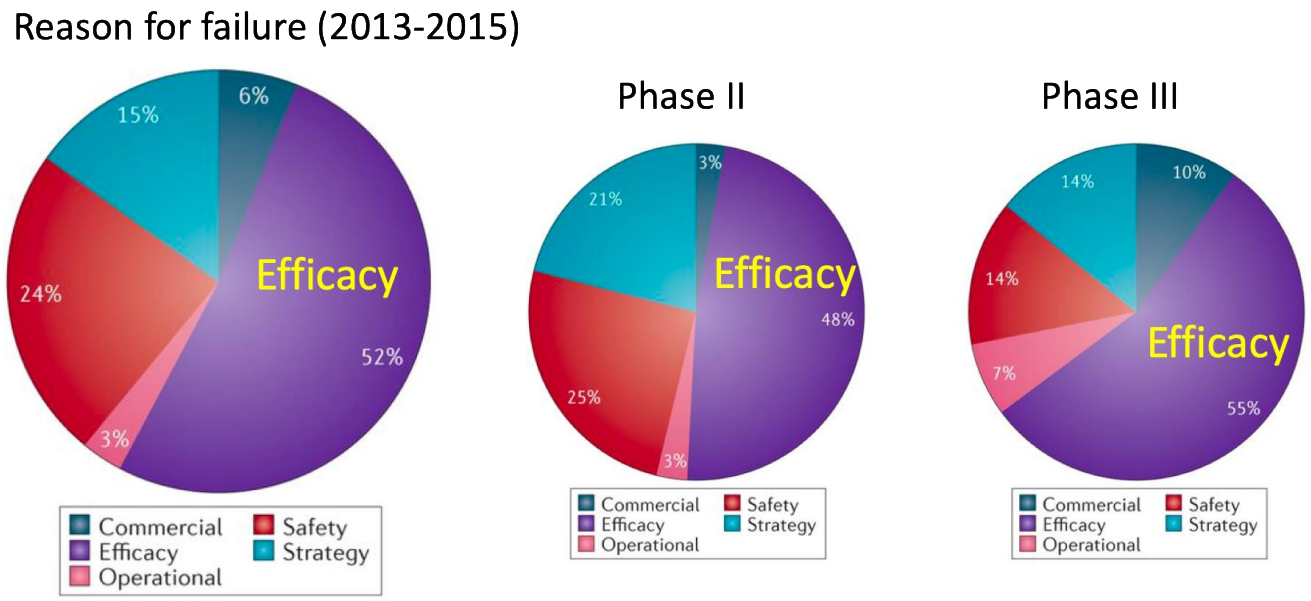
Any special considerations/precautions during the development of small-molecule drugs with TMDD?

Caution needs to be taken in;

- extrapolating the results from microdose to therapeutic dose; from a single dose to multiple doses
- designing cross-over studies (study intervals)
- comparing the data between patients and healthy volunteers (target levels may change and TMDD may be seen only in patients, potentially with interpatient variability)

Take-home Messages

- Small-molecule drugs may display **TMDD** characteristics (warfarin & other drugs with ***high potency & slow dissociation from abundant targets***).
- PBPK modeling of the systemic PK profiles over a wide dose range (below & at target saturation) may enable prediction of target occupancy profiles (key information for efficacy assessment).



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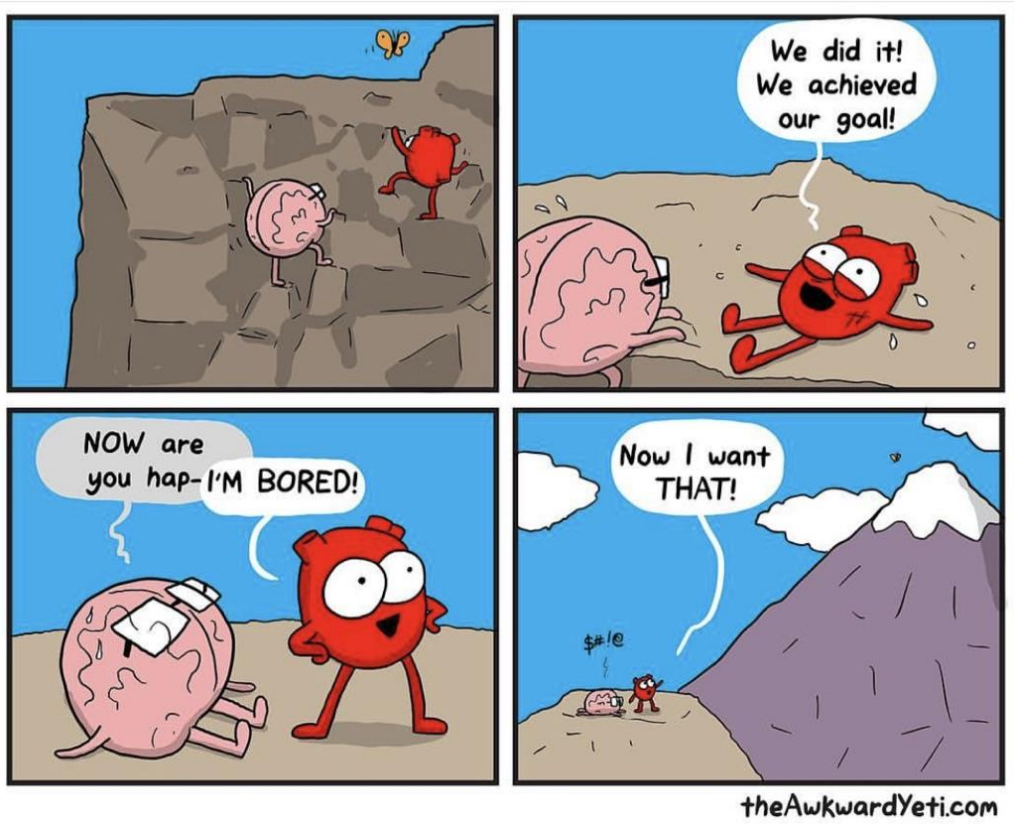

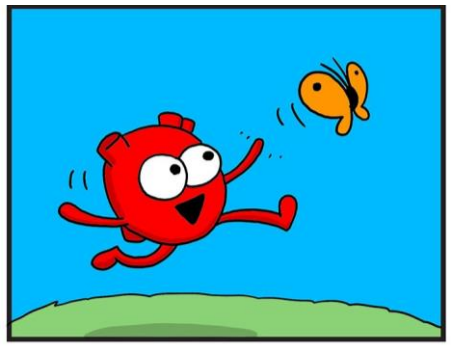

Anyone interested in climbing <small-molecule drugs with TMDD> mountains together??

Please contact **wooin.lee@snu.ac.kr**